Translation 540,165

PCT/JP2003/016653

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference B-346WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/JP2003/016653	International filing date (*day/month/year*) 25 December 2003 (25.12.2003)	Priority date (*day/month/year*) 26 December 2002 (26.12.2002)
International Patent Classification (IPC) or national classification and IPC C12N 9/12, C07H 21/02, C12P 19/34, C12N 15/54, 1/21 // (C12N 9/12, C12R 1:19)		
Applicant NIPPON SHINYAKU CO., LTD.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:

      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

   ☒ Box No. I Basis of the report
   ☐ Box No. II Priority
   ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   ☐ Box No. IV Lack of unity of invention
   ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   ☐ Box No. VI Certain documents cited
   ☐ Box No. VII Certain defects in the international application
   ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand 20 May 2004 (20.05.2004)	Date of completion of this report 15 November 2004 (15.11.2004)
Name and mailing address of the IPEA/JP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2003/016653

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is language of a translation furnished for the purpose of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ The international application as originally filed/furnished

☒ the description:

pages _____

pages* 1-15, as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____

pages* 1-7, as originally filed/furnished

pages* 10, as amended (together with any statement) under Article 19

pages* _____ received by this Authority on 14 October 2004 (14.10.2004)

pages* _____ received by this Authority on _____

☒ the drawings:

pages _____

pages* 1-6, as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☒ the claims, Nos. 8-9

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP 03/16653

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7, 10	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-7, 10	NO
Industrial applicability (IA)	Claims	1-7, 10	YES
	Claims		NO

2. Citations and explanations

Document 1: EP 1153931 A1 (Nippon Shinyaku Co., Ltd.), 14 November 2001

Document 2: US 4927755 A (Societe de Conseils de Recherches et d'Applications Scientifiques), 22 May 1990

Document 3 (additional): JP 5-219978 A (Yamasa Shoyu Kabushiki Kaisha) 31 August 1993, entire text (Family: none)

Document 4: J. Biol. Chem., 1987, 262 (1), pages 63 to 68 & Database GenBank accession No. J02638, December 20, 1995, Regnier, P. et al., *E. coli* rpsO and pnp genes encoding ribosomal protein S15 and polynucleotide phosphorylase, complete cds. & Database PIR accession No. H65106, March 01, 2002, Regnier, P. et al., polyribonucleotide nucleotidyltransferase (EC 2.7.7.8) alpha chain - *Escherichia coli* (strain K-12).

Document 5: Database GenBank accession No. AP002564, March 07, 2001, Ohnishi, M. et al., *Escherichia coli* 0157:H7 DNA, complete genome, section 15/20.

Document 6: J. Bacteriol, 1983, 154 (1), pages 58 to 64

Document 7: EP 1221478 A2 (National Food Research

Institute, et al.), 10 July 2002

Document 8: WO 98/36080 A1 (The Dow Chemical Company), 20 August 1998

Document 9: WO 99/57153 A1 (Insight Strategy & Marketing Ltd.), 11 November 1999

Document 10: EP 972836 A2 (The Institute of Physical & Chemical Research), 19 January 2000

Document 11: JP 9-23886 A (Wako Pure Chemical Industries, Ltd.), 28 January 1997

Document 12: WO 02/10370 A1 (Takeda Chemical Industries, Ltd.), 7 February 2002

Document 13: JP 2001-245666 A (Kyowa Hakko Kogyo Co., Ltd.), 11 September 2001

The invention set forth in claim 10 does not involve an inventive step in the light of documents 1 and 2 cited in the international search report and newly cited document 3.

Document 1 sets forth a method of producing synthetic nucleic acid polymers such as polyinosinic acid (1973 residue) and polycytidylic acid (3300 residue).

Document 2 indicates that a polynucleotide phosphorylase of *E. coli* origin is made to act on a nucleotide monomer such as CDP or IDP to obtain a polymer with a molecular weight of approximately 250,000 to 1,500,000. This molecular weight corresponds to residues of approximately 700 to 4000.

Document 3 indicates that polyinosinic acid and polycytidylic acid are manufactured using a polynucleotide phosphorylase of *E. coli* origin.

Documents 2 and 3 do not indicate that polynucleotide phosphorylase is manufactured using the production method set forth in claims 1 to 7, but the polynucleotide phosphorylase manufactured using the production method set forth in claims 1 to 7 and the

polynucleotide phosphorylase set forth in documents 2 and 3 are both polynucleotide phosphorylase or *E. coli* origin, and are identical, hence the disclose that "produced by the production method set forth in claims 1 to 7" is not acknowledged to specify PNPase.

In the light of the inventions set forth in documents 1 to 3, it would be easy for a person skilled in the art to conceive of producing polyinosinic acid and polycytidylic acid with a residue having a molecular weight falling within the approximate range of 700 to 4000 using a PNPase of *E. coli* origin. In addition, the numerical value giving a residue with an average chain length of approximately 2200 in the invention of this application is within the scope that a person skilled in the art could predict in document 2, therefore the invention set forth in this application does not offer a special and unexpected effect in the light of the inventions set forth in documents 1 to 3.

The invention set forth in claims 1, 5 to 7 and 10 does not involve an inventive step in the light of documents 1 to 10 cited in the international search report.

Documents 4 to 6 set forth a PNPase gene of *E. coli* origin such as strain K12 or strain 0157.

Documents 7 to 10 set forth a method wherein a gene which codes the target protein is integrated into plasmide having a T7 promoter, and said plasmide is used to transform and cultivate *E. coli* having a T7RNA polymerase gene to produce said target protein.

At the time of filing of this application, in the production of recombinant protein, when accumulating said recombinant protein in a transformant, it was a known technique to extract and refine said recombinant protein

from said transformant.

It would therefore be easy for a person skilled in the art to conceive of integrating a PNPase gene of *E. coli* origin such as strain K12 or strain O157 set forth in documents 4 to 6 to a plasmide having a T7 promoter, and using said plasmide transform and cultivate the *E. coli* having a T7RNA polymerase gene and extracting and refining PNPase from said transformed *E. coli*, and to prepare a synthetic nucleic acid polymer using said PNPase.

The invention set forth in claims 3 and 4 does not involve an inventive step in the light of documents 1 to 10.

At the time of filing of this application, in the production of recombinant protein it was a known technique to prepare a fused protein having a tag such as a His tag assigned to said protein.

The invention set forth in claim 2 does not involve an inventive step in the light of documents 1 to 13.

Documents 11 to 13 indicate that when producing recombinant protein with *E. coli* as a host, said *E.coli* is cultivated for between 3 and 24 hours or for between 16 and 96 hours.

The cultivation time in the production of recombinant protein is merely a design matter which would be optimized as necessary by a person skilled in the art, and it is generally acknowledged that if the cultivation period is set to a long period of time, a considerable percentage of the host will die and said recombinant protein will be accumulated outside the bacteria. Moreover, in producing recombinant protein, when accumulating said recombinant protein outside the transformant, it is a known technique to recover and refine said recombinant protein from the culture medium or

culture solution.

It would therefore be easy for a person skilled in the art to conceive of integrating a PNPase gene of *E. coli* origin such as strain K12 or strain O157 set forth in documents 4 to 6 to a plasmide having a T7 promoter; using said plasmide transform and cultivate for a long period of time the *E. coli* having a T7RNA polymerase gene; and extracting and refining PNPase from the culture medium and/or culture solution.